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## **RESEARCH**

### **New Cell Line Detects Endocrine Activity**

A new cell line has been developed by the National Health and Environmental Effects Research Laboratory (NHEERL) that may be useful in rapidly screening large numbers of chemicals to identify those with endocrine-disrupting properties. In recent years there has been a growing concern about the increasing number of chemicals shown to disrupt endocrine activity affecting the reproductive systems of humans or wildlife. Protocols are being developed by the Reproductive Toxicology Division for the screening of chemicals that are potentially endocrine-active. Led by Dr. Vickie Wilson and Kathy Bobseine, research to develop the new cell line is published in the March 2002 issue of *Toxicological Sciences*.

A breast cancer cell line, MDA-MB-453, was used as a starting point. These immortal cells contain binding sites for androgenic (male) hormones. When the cells were treated to alter their DNA, they were transformed into a new cell line called MDA-kb2. The change in DNA remained stable over many generations of cells grown from this line. Subsequent exposure of the new cell line to chemicals with male hormone-like properties induces a change at the binding sites that can be measured rapidly and quantitatively.

The MDA-kb2 cell line will be made available to the American Type Culture Collection, which guarantees safekeeping and distributes cell line samples to researchers worldwide at a nominal charge. This cell line has the potential to be used to screen large numbers of chemicals and to prioritize the use of more expensive and time-consuming in vivo assays using animals.

## **Competition for Resources Influences Plant Diversity**

A clearer view of how plants compete for resources and how this competition influences the species composition of a community is provided in a paper published in the Jan. 3, 2002, issue of the journal *Nature* by Dr. Robert McKane of the Western Ecology Division (WED), the lead author of 11 investigators. The paper documents, for the first time, that species diversity and dominance in a plant community is closely linked to how the plants divide a single limiting below-ground resource—nitrogen, in this case.

The field work and laboratory analyses were funded by a grant from the National Science Foundation when McKane was employed at the Marine Biological Laboratory in Woods Hole, Massachusetts, but he did the data analyses and wrote the paper after joining WED in Corvallis, Oregon.

At Toolik Lake, near Alaska's Arctic National Wildlife Refuge, the researchers studied tussock tundra plants whose growth is limited primarily by the availability of nitrogen in the soil. They found that the plants in this community took up nitrogen at different times, depths, and in different forms. They also found that the most productive plant species, cottongrass, used the most abundant forms of nitrogen, while less productive species used less abundant forms. Cottongrass is primary forage for caribou and other arctic wildlife, but has declined in abundance in recent decades. The decline coincides with recent increases in arctic temperatures that may alter the availability of different forms of soil nitrogen.

Ecologists have long been interested in how species living in the same community divide limited resources and thereby promote diversity by reducing competition. This resource partitioning, or niche differentiation, is known to be an important determinant of species diversity and composition in animal communities. However, its importance in structuring plant communities has been difficult to determine,

primarily because of difficulties in measuring how plants compete for below-ground resources.

The researchers overcame that problem by performing more than 15,000 injections of tundra plots with  $^{15}\text{N}$ , a stable isotope of nitrogen, and analyzing more than 2,000 plant samples for isotope uptake. By injecting different chemical forms of  $^{15}\text{N}$  at different times and soil depths, they discovered how plants took up naturally occurring forms of soil nitrogen. The study provides a clearer view of below-ground competitive interactions than existed previously, and establishes a means to better address fundamental questions about plant species diversity.

### **Bromoacetic Acid Affects Male Reproduction and Fertility in Rats**

The haloacid, bromoacetic acid (BCA), one of the more prevalent disinfection by-products (DBPs) that can form when chlorine is used in water to kill pathogens, has been found to cause adverse effects on sperm production in rats. The Reproductive Toxicology Division (RTD) studied the noncancer effects of BCA on reproduction in male rats and compared the results to earlier work on another prevalent DBP, dibromoacetic acid (DBA), which showed that treating adult male rats produced adverse effects on sperm production.

Scientists reported that BCA, like DBA, also adversely affected the formation of normal sperm and the fertility of treated male rats. Fertility was decreased about 25-30 percent at all BCA doses used, even the lowest one. These results are published in the January/February 2002 issue of the *Journal of Andrology*, where they are also featured in photographs on the cover.

The fertility of treated rats was also compared to the level of a specific sperm protein, SP22, found on the surface of sperm. A decrease in the level of SP22 was present in all dosage groups and was correlated with a decrease in fertility in treated

rats. The study confirmed that SP22, a protein identified earlier by RTD, is a useful biomarker for fertility, although its application in assessing human infertility is as yet unknown.

These results suggest that reproductive alterations in male rats are likely even at much lower dosage levels. Therefore it is important to determine additivity effects associated with exposure to low-dose mixtures of haloacids, a condition that better represents how humans are exposed. Future studies at RTD will assess whether effects on SP22 and fertility of rats are additive when low-dose mixtures of haloacids are used. Under a Cooperative Agreement with the University of North Carolina at Chapel Hill, an epidemiology study is looking at the effects on the fertility of sperm from men drinking water with varying levels of DBPs; SP22 will be one of the sperm parameters measured. Other Cooperative Agreements with Research Triangle Institute and Colorado State University are studying fertility and SP22 levels in male offspring of mother rats exposed during pregnancy to low doses of BCA and DBA, respectively. The results of all of these studies will contribute to decisions on whether the Maximum Contaminant Level for haloacids is protective enough for human exposure.

### **Heptachlor Exposure Causes Subtle, Persistent Deficits in Rats**

Two recent publications by scientists in the Neuortotoxicology Division (NTD) revealed subtle but long-lasting behavioral and neurological effects of the cyclodiene pesticide heptachlor on rat development. Although heptachlor was banned in the 1980s, it is still found in food samples and is considered a “persistent organic pollutant” by the United Nations Environment Programme. These results are particularly relevant because other cyclodiene pesticides are in restricted commercial use today. The research was partially supported by the Hawaiian Heptachlor Research and Education Foundation from a trust fund established after routine monitoring discovered high exposure to heptachlor from contaminated cow’s milk on the island of Oahu in the 1980s.

Using doses similar to levels found in human breast milk samples taken in 1981 in Oahu, rats were exposed to heptachlor before birth and for several weeks after birth. As described in the April 2001 issue of *Toxicological Sciences*, NTD researchers found a delay in behavioral development in young rats and, later, an impairment of a learning and memory task when those rats reached adulthood.

Also investigated was the possibility of increased susceptibility to neurodegenerative diseases after developmental exposure to heptachlor. The doses used were no more than 10-fold greater than those in the behavioral study, and were used over a shorter dosing period. As published in the December 2001 issue of *Toxicological Sciences*, rats treated with heptachlor during early stages of development showed neurochemical changes as adults. They had increased binding to the dopamine transporter (DAT) receptor sites, implying an increase in receptor levels. The DAT allows entry of other neurotoxicants into neurons, and may also be involved in maintaining neuronal integrity. Heptachlor exposure during neuronal development increased DAT binding during early development, adolescence, and adulthood. Similar results were obtained with dieldrin, another cyclodiene pesticide.

It is hypothesized that these neurochemical changes may increase susceptibility to other neurotoxicants, which may cause degenerative diseases, such as Parkinson's disease, later in life. Further research is critical to understanding the potential influences of developmental toxicant exposure on neurological and degenerative outcomes.

### **Relationship of Dioxin Exposure to Sanitary Products and Endometriosis Studied**

Two papers by NHEERL scientists in the January 2002 issue of *Environmental Health Perspectives* address whether sanitary products are a source of dioxin and whether endometriosis is associated with dioxin exposure. Dioxins are a family of long-lasting, bioaccumulating, toxic chemicals found in the environment largely as a result of

manufacturing and combustion processes. This family, which includes some polychlorinated dibenzodioxins, dibenzofurans, and biphenyls, is known to affect human and animal health in many ways, including effects on the endocrine and immune systems. The most toxic member, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or “dioxin”), is frequently used as a reference compound in toxicity studies.

There is particular concern about possible effects on infants and children from exposure to dioxins in diapers. The media also have raised concern about possible links between dioxin exposure from tampons causing endometriosis, a disease of the reproductive system that affects millions of women in the United States.

A study published by Dr. Michael DeVito of the Experimental Toxicology Division, in collaboration with Dr Arnold Schechter of the Environmental Sciences Discipline, University of Texas School of Public Health at Dallas, concluded that although exceedingly low concentrations of dioxins were found in the diapers and tampons sampled, these sanitary products made in the United States do not contribute significantly to dioxin exposure. For comparison, the estimated dietary exposure of infants to dioxins is approximately 30,000 to 2,200,000 times greater than their exposure from diapers. The estimated dietary exposure of women to dioxins is approximately 13,000 to 240,000 times greater than from tampons.

In the second paper Dr. Linda Birnbaum of the Experimental Toxicology Division and Dr. Audrey Cummings of the Reproductive Toxicology Division critically reviewed the literature of the past nine years on the relationship between exposure to dioxins and an increased incidence of endometriosis, and they suggest a possible connection. But the hypothesis that dioxins are causally related to endometriosis needs further study.

Studies on monkeys and rodents have linked exposure to TCDD and other dioxins to an increased incidence or promotion of endometriotic lesions. Recent epidemiological studies also suggest a link between these chemicals and the incidence



of the disease in humans, but these conclusions remain controversial because there is insufficient relevant information in the literature on which to base a definitive judgment.

### **Study Launched on How Microorganisms Cause Gastroenteritis**

A study on gastrointestinal disease in Texas is part of a large research effort by the Human Studies Division to determine the extent and causes of waterborne disease in the United States. On average, adults in the United States have one to two episodes a year of gastroenteritis (diarrhea and vomiting), but young children and elderly adults have six to 10. Although most cases are caused by any of a wide variety of microorganisms, how people come in contact with these disease-causing agents is rarely known. The Southwest Texas Enteric Epidemiology Study, sponsored by EPA and starting in March 2002, will attempt to determine how microorganisms are transmitted to humans and cause gastrointestinal disease. Dr. Rebecca Calderon of the Human Studies Division is the EPA Project Officer.

The Texas study will determine the incidence of gastrointestinal illnesses in two areas that get their drinking water from different sources. Participants in the Eagle Pass area use Rio Grande water for drinking, whereas participants in the Del Rio area use spring water. Up to 150 families in each of the two communities will be studied. The families will record their own episodes of gastroenteritis, as well as contacts with infected persons, food, drinking water, recreational water (such as swimming and wading pools), and animals, all potential risk factors for gastroenteritis. From these data the researchers will be able to estimate how much gastrointestinal illness is associated with each potential source of infection.

Data collection will be completed by the summer of 2003, and preliminary results are expected by December 2003. Field work on similar studies in Massachusetts and Washington has been completed, and one in Iowa will conclude this spring. Preliminary results are expected by the end of 2002. Data from these studies will be used to make

national estimates of waterborne disease, as mandated by the Safe Drinking Water Act Amendments of 1996.

### **Postdoc Co-Authors Three Papers on Toxicogenomics**

Understanding the development of cancer cells in humans and animals is the focus of three papers on toxicogenomics. Extending work done under the mentorship of Dr. Anthony DeAngelo of the Environmental Carcinogenesis Division (ECD), Dr. Lynn Crosby co-authored three articles on toxicogenomics recently accepted for publication. She is an EPA Postdoctoral Fellow in ECD from the Curriculum in Toxicology at the University of North Carolina at Chapel Hill. Other authors are from the National Institutes of Environmental Health Sciences, Duke University Medical School, and GlaxoSmithKline Inc., all in North Carolina's Research Triangle.

The emerging field of toxicogenomics studies the response of cells to stress at the most basic level: the molecules within the cell. The cell may respond by adaptations such as DNA repair with accompanying temporary growth cessation; the production of molecules that counteract the stimulus, such as cytokines that fight infection; or cell death.

One paper, to be published in the July/August 2002 issue of *Toxicologic Pathology*, describes the development of a 7-gene oxidative stress test on cultures of human liver cancer cells. Because oxidative stress is a consequence, and possibly also a cause, of many adverse health outcomes, including Alzheimer's, Parkinson's disease, and cancer, this relatively quick, efficient, and cost-effective screen to detect the oxidative stress potential of various substances will benefit many stakeholders.

Related review articles, published in the January/February 2002 issue of *Toxicologic Pathology* and to be published in a spring 2002 issue of *Human and*

*Ecological Risk Assessment*, address the role of toxicogenomics in pathology and drug discovery and in risk assessment, respectively.

## ***TECHNICAL ASSISTANCE***

### **EMAP Technology Transferred at Coastal Assessment Workshop**

As part of EPA's strategic partnership with coastal states, the Gulf Ecology Division held a National Coastal Assessment workshop Jan. 29-30, 2002 in Gulf Breeze, Florida, for 14 representatives from South Carolina, Georgia, Florida, Alabama, and Mississippi. The National Coastal Assessment (formerly Coastal 2000) is a large-scale, comprehensive environmental monitoring program that characterizes the Nation's coastal resources—both estuaries and offshore waters.

The National Coastal Assessment program is based on EPA's Environmental Monitoring and Assessment Program that uses Geographic Information System technology to select sampling sites. Base sites for the first year's monitoring were distributed throughout the 24 coastal states and Puerto Rico. Each State or Commonwealth had at least 35 randomly selected sites that were sampled by its own resource agency personnel who had already completed the rigorous field sampling training offered by EPA.

The workshop transferred EPA-developed technology and expertise to the States and ensured their complete competency in implementing the program at, for, and by their own States. Topics covered included the development of assessment questions, conceptual models, sampling and response designs, quality assurance, data analysis and interpretation, information management, and reporting. Similar workshops are being planned for other States, to cover the entire coastline of the United States.

## ***MEETINGS / CONFERENCES***

### **EMAP Symposium 2002 Coming May 7 - 9**

“EMAP Symposium 2002: The Conditions of Our Nation’s Streams & Rivers from the Mountains to the Coasts” will be held May 7 - 9 in Kansas City, Missouri. More than 250 participants from Federal, State, and local governments, tribes, universities, and nonprofit organizations across the country are expected to attend.

Co-sponsored by EPA’s Office of Research and Development and by the Council of State Governments, the symposium will be one of the major events of EPA’s Environmental Science Month in May. The principles and procedures developed in EPA’s Environmental Monitoring and Assessment Program (EMAP) will be applied directly to help States and tribes evaluate the quality of their streams and rivers. Because EMAP has already been used successfully in the East and a major effort is currently under way on western streams, it now can be applied to inland surface waters. Hence the location of the symposium in America’s heartland. Later, EMAP procedures will be applied to the great rivers of the Midwest—the Mississippi, Missouri, and Ohio.

Four themes are used to organize the symposium:

- Monitoring Streams and Rivers of the U.S.: EMAP-West and State Studies
- Using EMAP to Create the Big Picture of Great Rivers
- Integrating 305(b) and 303(d) of the National Water Quality Inventory Report: How EMAP Aids in Monitoring and Assessment of State Waters
- Socioeconomic Monitoring and Assessment

For additional information, visit the symposium Web site at [http://www.csg.org/emap\\_symposium\\_2002.htm](http://www.csg.org/emap_symposium_2002.htm) .

## ***PUBLICATIONS***

### **Atlas Provides Scientific Basis for Land Use Planning**

An atlas that charts environmental impacts for different development scenarios has been completed after an eight-year collaborative research project that brought together Federal, State, and university scientists to address land-use implications in Oregon.

Since 1994, the Pacific Northwest Ecosystem Research Consortium, an umbrella organization including the Western Ecology Division (WED), the University of Oregon, Oregon State University, and the University of Washington, has been studying Oregon's Willamette River Basin in great detail, not just at the local level, but at the much broader landscape level. Their research is culminating this spring in the publication of a 178-page atlas that charts differing environmental futures for the basin. The large-format atlas, "Willamette River Basin: Trajectories of Environmental and Ecological Change," is being published by Oregon State University Press.

Dr. Joan Baker of WED was the EPA Project Officer; she is also a co-editor of the atlas. The information provided in the atlas will allow the region's residents to understand the implications and consequences of land-use and development decisions.

The atlas has three sections. The first covers base data on landforms, water resources, biotic systems, human population, and land use and land cover. The next section describes three potential alternative futures for this region of 2 million residents, and makes projections for each alternative to the year 2050, by which time the



population is expected to double. Finally, there is an extensive section on potentials for restoration of the Willamette River.

Alternative futures involve three different scenarios for development: one opting for extensive conservation measures, one pursuing increased rates of development, and one following existing state and local development guidelines. The atlas describes the likely effects of each of these future landscape changes on stream condition, wildlife, and water availability and use, relative both to current and historical conditions in the Willamette Basin.

### **Dioxin Toxicity Equivalence Factors in Fish and Wildlife Published**

Scientists have known for years that dioxin is harmful to humans, fish, and other wildlife. The most potent and intensively studied dioxin, TCDD (2,3,7,8-tetrachloro-dibenzo-*p*-dioxin), is used as the basis or reference compound for determining the toxicity of other compounds with dioxin-like properties. A scientific collaboration has advanced the development of assessment tools for determining the toxicity of environmental chemicals.

Three NHEERL scientists (Dr. Philip Cook of the Mid-Continent Ecology Division and Drs. Michael DeVito and Tala Henry of the Experimental Toxicology Division) were key contributors to a recently completed 385-page report on a 1998 workshop on toxicity equivalence factors (TEFs) used to compare the toxicity levels of various dioxin-like compounds. The report, "Workshop Report on the Application of 2,3,7,8-TCDD Toxicity Equivalence Factors to Fish and Wildlife" (EPA/630/R-01/002) is available on the Web at <http://www.epa.gov/ncea/raf/rafpub.htm> .

A TEF estimates the potency of an individual polychlorinated dioxin, furan, or biphenyl to cause dioxin-like effects. The publication is part of a broader effort of EPA's

Risk Assessment Forum on how to assess ecological risks posed by mixtures of dioxin-like compounds to fish and wildlife.

Sponsored by EPA and the Department of Interior, the workshop was a major step in jointly addressing the possibility of applying TEFs to the Great Lakes Water Quality Guidance. The use of TEFs and the toxicity equivalence approach in ecological risk assessment was strongly endorsed by workshop participants. As a result, the NHEERL authors now work with other scientists from EPA's Regional Offices and Risk Assessment Forum, the U.S. Fish and Wildlife Service, and the U.S. Geological Survey to address issues with multi-disciplinary and cross-program complexities in more depth than in the report. This work is part of an ongoing effort by the forum to develop a framework for applying the toxicity equivalence methodology in ecological risk assessment.